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YALE JOURNAL OF BIOLOGY AND MEDICINE 89 (2016), pp.513-525.

REVIEW

YJBM

The Role of the Ubiquitously Expressed Transcription Factor Sp1 in Tissue-specific Transcriptional Regulation and in Disease

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Sp1 belongs to the 26 member strong Sp/KLF family of transcription factors. It is a paradigm for a ubiquitously expressed transcription factor and is involved in regulating the expression of genes associated with a wide range of cellular processes in mammalian cells. Sp1 can interact with a range of proteins, including other transcription factors, members of the transcription initiation complex and epigenetic regulators, enabling tight regulation of its target genes. In this review, we discuss the mechanisms involved in Sp1-mediated transcriptional regulation, as well as how a ubiquitous transcription factor can be involved in establishing a tissue-specific pattern of gene expression and mechanisms by which its activity may be regulated. We also consider the role of Sp1 in human diseases, such as cancer.

INTRODUCTION

Gene expression needs to be tightly regulated as the specific pattern of gene activation or repression is decisive for establishing fates. The gene expression program of a cell is controlled by the activities and the interactions of the epigenetic regulatory machinery and sequence-specific transcription factors. The epigenetic machinery consists of enzymes that post-translationally modify histone proteins, such as histone acetyltransferases (HATs†), histone deacetylases (HDACs), histone kinases and methyltransferases, as well as ATP-dependent chromatin remodeling complexes [1]. These factors regulate gene expression by altering the conformation of DNA and allowing access to key regulatory elements of transcription. Transcription factors bind to specific regulatory sequences in the DNA and regulate transcription of the associated gene by promoting recruitment of the transcription initiation machinery. Additionally, transcription factors are capable of directing histone modifying enzymes and chromatin remodeling complexes to specific sites, such as gene promoters, thus preparing the gene for transcription or, in the case of repressors, blocking it.

Transcription factors interact in a combinatorial fashion to uniquely regulate genes and, in response to different stimuli, regulate tissue-specific and developmental stage-specific gene expression. Many transcription factors are expressed in a tissue-specific manner and regulate the specialized functions of a particular cell; therefore elimination of these factors can result in a block in development/differentiation. For example, SCL/TAL1 is a crucial transcription factor in the hematopoietic system and the deletion of its gene in mice results in a failure to generate hematopoietic precursors and embryonic death [2]. Other transcription factors are ubiquitously expressed and are generally involved in the expression of ubiquitously expressed "housekeeping" genes in all cell types. However, they can also interact with tissue-specific proteins or be post-translationally modified in a tissue-specific manner to elicit a particular pattern of gene expression. Nuclear Factor I (NFI) family members are ubiquitously expressed and are involved in the regulation of constitutive genes and those that are controlled by hor-

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†Abbreviations: AML, acute myeloid leukemia; BTD, Buttonhead domain; DNMT, DNA methyltransferase; EMSA, electrophoretic mobility shift assay; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ESC, embryonic stem cell; HAT, histone acetyltransferase; HCC, Hepatocellular carcinoma; HDAC, histone deacetylase; Htt, Huntingtin; KLF, Krüppel-like factor; MS, multiple sclerosis; NFI, Nuclear Factor I; Sp, Specificity protein; TAF, TBP-associated factors; TBP, TATA-binding protein; TK, Thymidine Kinase.

Keywords: Sp1 transcription factor, gene regulation, tissue specificity, human disease, epigenetics

monal, nutritional, and developmental signaling [3]. However, the knockout of individual NFI members results in tissue-specific defects. For example, NFI-A knockout mice have a defect in brain development (formation of the corpus callosum) but few other anatomical problems [4]. This suggests that the four NFI family members have overlapping roles and can compensate for the lack of NFI-A activity, but there are distinct functions in specific tissues, revealed by the defects, indicating ubiquitous transcription factors have important roles in development and tissue-specific gene expression. Conversely, knockout of the gene encoding OCT1, another ubiquitously expressed transcription factor, leads to developmental arrest at a very early embryonic stage [5], suggesting widespread roles. Although OCT1 is important in the expression of housekeeping genes, such as *H2B* [6], it also activates tissue-specific genes, often via interactions with cell-specific proteins [7-9]. The *IL3* locus is an example of such a target. T Cell Receptor signaling leads to activation of an inducible T cell-specific enhancer upstream of the *IL3* gene, containing NFAT and OCT1 binding sites. Studies have shown the two transcription factors interact at the enhancer to synergistically activate T cell-specific *IL3* expression [8]. Importantly, this system demonstrates how cooperation with a cell-specific protein can alter the binding or the activity of a ubiquitous transcription factor to bring about tissue-specific gene expression.

Sp1 is a transcription factor that has been found to be present in all mammalian cell types [10]. Thus, it was long thought to be solely a regulator of housekeeping genes and indeed, knockout of Sp1 in mice causes embryonic lethality at an early stage of development (around day 10.5 of gestation) with a broad range of phenotypic abnormalities, suggesting a general function in many cell types [11]. However, Sp1 is now also known to be involved in the regulation of tissue-specific, cell cycle, and signaling pathway response genes [12], with chromosome mapping studies estimating there are at least 12,000 Sp1 binding sites in the human genome, associated with genes involved in most cellular processes [13]. Furthermore, its expression levels were seen to vary in different cell types and through different stages of mouse development [14], and it is required for the transcriptional activation of Hsp70.1, one of the first genes expressed after fertilization in mouse embryos [15], highlighting Sp1's importance in development. It has also been shown to play a role in numerous human diseases, including cancer. Therefore, it is important to fully understand Sp1's mode of action and contribution to gene regulation.

THE SP/KLF FAMILY

Sp1 was the first mammalian transcription factor to be cloned and characterized, named originally according to the purification procedure used (Sephacryl and phosphocellulose columns), but now more commonly named Specificity protein 1 [16,17]. When whole cell extracts were prepared from HeLa cells to study the factors required for transcription initiation *in vitro*, Dynan and Tjian identified that one of these factors, Sp1, showed sequence specificity and was able to bind to the SV40 early promoter and activate transcription of the gene [18].

Sp1 is the founding member of the Specificity protein/Krüppel-like factor (Sp/KLF) family of transcription factors, which currently has a total of 26 members [19]. The family is characterized by the highly conserved DNA binding domain (sequence identity > 65 percent) near to the C-terminus of all members, which recognize GC (consensus sequence: GGGGCGGGG), as well as GT/CACC (GGTGTGGGG) boxes [16,20-22]. The DNA binding domain is made up of three adjacent Cys2His2-type zinc fingers consisting of exactly 81 amino acids in every protein [17]. Not only are the amino acids within the individual zinc finger structures conserved, but there are also constraints on the residues in the interfinger regions, with the conserved linker sequence T/S-G-Q-R/K-P, suggesting the zinc fingers act as a single unit [23]. The residues that are directly in contact with the DNA, and therefore providing the specific base recognition, are the most conserved parts of the protein. In Sp1, these residues were identified as KHA in the first zinc finger, RER in the second and RHK in the third, although there are slight changes in some of the other family members that correspond with differing preference to GT boxes rather than GC boxes, or differing binding affinities [16,24].

The Sp/KLF family is split into two groups based on the structure at the N-terminus: Sp-like transcription factors (Sp1-9) (Figure 1) and the KLF-like transcription factors, named from the Cys2His2 zinc finger Krüppel protein in *Drosophila*. In general, the Sp-like family recognize GC boxes in preference to GT boxes, while the reverse is found for the KLF-like family, which comprises both transcriptional activator and repressor proteins. In terms of structure, the nine Sp-like members are distinguished from the KLF-like proteins by the presence of a conserved Buttonhead domain (BTD, first identified in the *Drosophila* Sp1 homologue Buttonhead) N-terminal to the DNA binding domain [23,25]. Its function is debated, but studies suggest it is involved in the transactivation or synergistic activities of the Sp proteins [26,27]. Another feature in most Sp-like proteins is a conserved stretch of amino acids at the N-terminus of the protein with the sequence SPLALLAATCSR/KI, termed the Sp box [23]. Again, the precise function of this motif is unknown, but as it contains an endoproteolytic cleavage site and is located close to the region in Sp1 that targets proteasome-dependent cleavage [28], one theory is that it may have a function in regulation of protein degradation.

The Sp-like protein family can be further subdivided into Sp1-4 and Sp5-9, with Sp1-4 being distinguishable by the presence of N-terminal glutamine-rich transcriptional activation domains. Overall, Sp1-4 have a very sim-

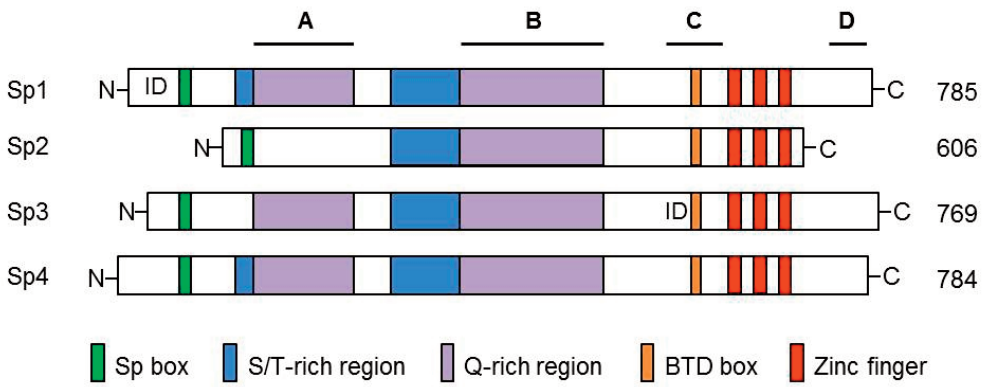

Figure 1. Primary structure of the Sp-like transcription factors.
Conserved domains of the Sp-like transcription factors are illustrated. Two glutamine (Q)-rich domains (A and B) form the transactivation domains, while the inhibitory domains (ID) present in Sp1 and Sp3 are also indicated. Three zinc fingers at the C-terminus comprise the DNA binding domain and domain C contains a highly charged region adjacent to the DNA binding domain. Domain D in Sp1 enables synergistic binding with other Sp1 proteins. The conserved sequence motifs, BTD and Sp boxes, are also shown and the length of the protein in amino acids is indicated on the right.

ilar modular domain structure with Sp1, Sp3 and Sp4 being more closely related in structure and activity than Sp2. The former proteins have a highly conserved DNA binding domain sequence and bind to GC boxes (and to a lesser extent, GT boxes) with similar affinities. Sp2, however, preferentially binds GT boxes due to changes from the consensus zinc finger DNA-binding residues, with a leucine substituted for the conserved histidine residue in the first zinc finger [21,29]. Sp1, Sp3, and Sp4 each contain two glutamine-rich transactivation domains, termed A and B, located near to a serine/threonine-rich sequence, which is the target of many posttranslational modifications. Sp2, on the other hand, only contains one glutamine-rich domain, but they share a highly charged region adjacent to the DNA binding domain (domain C) [21].

Both Sp1 and Sp3 are ubiquitously expressed in mammalian cells. They have highly similar structures, with their DNA binding domains sharing over 90 percent DNA sequence homology, meaning that the two transcription factors recognize the same DNA sequence element and bind with similar affinity [21,30]. However, while knockout of Sp1 in mice causes death at around day 10.5 of gestation [11], Sp3 knockout mice die postnatal, apparently of respiratory failure [31,32]. In addition, mice heterozygous for either transcription factor appear normal, albeit slightly smaller, but being heterozygous for both proteins leads to embryonic lethality with a diverse range of phenotypes [32]. This suggests that, despite having very similar DNA specificity and affinity, the two transcription factors perform distinct functions in the cell. In support, high resolution fluorescent microscopy studying immunolocalization of Sp1 and Sp3 in the MCF-7 cell line revealed that both proteins were concentrated in discrete regions of the nucleus and are part of different promoter complexes [33]. However, the expression of many Sp target genes in *Sp1*$^{-/-}$ mice was found to be unaffected, suggesting that Sp3 may be able to compensate, in part, for loss of Sp1-mediated transcription and thus there is potential redundancy between Sp1 and Sp3 functions [11].

The biggest differences between the structures of Sp1 and Sp3 are a) the presence of a domain D at the C-terminal end of Sp1 only (important for synergy and multimerization, see below) [34] and b) the position of the inhibitory domain, which suppresses the transcription activation potential. In Sp1, the inhibitory domain is located at the N-terminus and acts by interacting with co-repressor molecules [35], while in Sp3, it is positioned just N-terminal to the three zinc fingers [36]. Transfection of Sp3 into *Drosophila* SL2 cells showed that it could only activate a portion of Sp target promoters and could not activate reporter gene constructs [37]. Mutation analysis identified the inhibitory domain, which worked to silence the two transactivation domains, and highlighted the importance of a highly charged amino acid motif 'KEE' for inhibitory behavior. Mutation of the KEE motif to alanine residues converted Sp3 into a strong transcriptional activator, identifying a means by which Sp3 activity could be regulated [36]. It is theorized that these structural differences are responsible for the functional differences of Sp1 and Sp3 [29,31].

The functions of other Sp proteins have also been studied. Expression of Sp2 is detectable in embryonic stem cells (ESCs) and in all tissues during embryogenesis (except the heart). It was initially found to be unable to stimulate transcription from promoters that are activated by other Sp members [38], but knockout of Sp2 in mice led to embryonic death at day 9.5, showing it is an essential transcription factor in mouse development [39]. Sp4 knockout mice show no obvious abnormalities, but two thirds die within their first month and the surviving mice

are smaller in size with abnormal reproductive organs [40,41]. Overall, Sp4 knockout studies suggest that the transcription factor is required for specification of the cardiac conduction system [42] and normal brain development [43]. Knockout of members of the KLF family have also been found to be embryonic lethal (KLF1, 2, 5 and 6), with the others displaying abnormalities in a range of tissues [44]. These deletion studies show the importance of the Sp/KLF transcription factors, while the variety of phenotypes shows that despite their structural similarity, they have distinct functions.

SP1-MEDIATED TRANSCRIPTION REGULATION

The Sp-like family of transcription factors generally function to activate transcription, whereas the KLF-like subgroup contains both activators and repressors of gene expression. Sp1 functions by recruiting the basal transcription machinery and, specifically, interacting with members of the TFiiD complex. TFiiD is composed of the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs). It is the first component of the transcription machinery to bind to the promoter, which then triggers formation of the pre-initiation complex. These interactions are known to be regulated by various transcription activators [45]. An *in vitro* transcription system first revealed that TFiiD was necessary and sufficient for Sp1 mediated transcription [46]. Characterization of the components of the TFiiD complex showed that the *Drosophila* protein dTAFii110 (the human homologue is hTAFii130) contained both glutamine- and serine/threonine-rich domains, similar to that of Sp1, leading to the hypothesis that the two proteins were functionally linked. Indeed, a yeast two hybrid assay demonstrated interactions between the transactivation domains of Sp1 and the N-terminus of dTAFii110, and transcription activation by Sp1 was increased with the addition of the dTAFii110 N-terminal fragment [46]. Deletion mutants revealed that each Sp1 transactivation domain interacts with distinct regions of hTAFii130. Of the four glutamine-rich domains (Q1 to Q4) within hTAFii130, the interaction with transactivation domain B of Sp1 was only disrupted upon deletion of Q1, whereas transactivation domain A made multiple contacts to hTAFii130 at Q2, Q3 and Q4 [47]. Interestingly, it was found that it was the hydrophobic residues within the transactivation domains that were important for the binding to dTAFii110 and subsequent transcription activation, whereas mutation of the glutamine residues had no effect [48]. These studies were then followed by the discovery that Sp1 also binds to TBP with the interaction occurring via the Sp1 glutamine-rich transactivation domains and the conserved C-terminus of TBP. There was a correlation between the extent of binding and the level of transcription *in vitro*, but TBP alone was not sufficient to activate Sp1-mediated transcription [45]. Therefore, Sp1 regulates transcription by communication with the RNA polymerase II transcription machinery.

Sp1 also regulates gene expression by affecting the chromatin state. It has been found to interact with histone modifying enzymes, including the HAT p300 [1,49]. The interaction between the DNA binding domain of Sp1 and the acetyltransferase catalytic domain of p300 leads to increased binding of Sp1 to DNA. Despite the DNA binding domain of Sp1 being acetylated by p300 during the interaction, there was little effect on DNA binding under varying acetylation conditions, suggesting that the increase in DNA binding was due to the direct interaction of the two proteins [49]. Thus, gene expression can be promoted both by changing the chromatin modifications at the promoter towards a more permissive structure and by the binding of a transcription activator, in this case Sp1. DNA binding of Sp1 then causes a release of p300, allowing it to regulate expression at further genes [49]. An example of cooperation between Sp1 and p300 is found during neuronal differentiation (in response to nerve growth factor signaling) when they activate the *p21* promoter to bring about withdrawal of the progenitor neural cells from the cell cycle [50].

Sp1 can also interact with negative epigenetic modifiers to cause down-regulation of gene expression [12,33]. Trichostatin A, an HDAC inhibitor molecule, was shown to lead to activation of the Thymidine Kinase (*TK*) gene. This gene is a target of Sp1 and indeed, co-immunoprecipitation experiments showed HDAC1 and Sp1 to be part of the same complex with interactions occurring via the C-terminal DNA binding domain of Sp1. The presence of Sp1 was also required for HDAC1-mediated *TK* repression, showing that Sp1 is involved in HDAC-mediated transcription inhibition [51]. HDAC1 was similarly found to be associated with Sp1 at the *p21* promoter in proliferating cells, so regulating cell cycle progression [52]. In addition, the DNA methyltransferase DNMT1 was found to bind to Sp1 and elicit repression of some Sp1 target genes, such as *MAZ* [53]. DNMT1 bound to seven consensus amino acids in the N-terminus of Sp1 and, at the Survivin promoter, were found to act together to inhibit gene expression (in response to p53 signaling and cell stress). In addition, Sp1 gradually recruited other transcriptional repressors, such as HDAC1 [54], to control gene expression in response to changing cell conditions. In contrast, Sp1 is involved in the maintenance of a methylation free state at the CpG islands in target gene promoters, for example at the *APRT* gene [55,56]. The methylation free CpG islands on *APRT* corresponded to three GC boxes, which footprinting revealed were bound by Sp1. The promoter region became methylated upon deletion or mutagenesis of the Sp1 binding sites, suggesting that Sp1 sites are required for the maintenance of CpG islands and the activation of gene expression [56]. Similar interactions with epigenetic regulators have also been reported for Sp3 [57-59].

Sp1 is capable of synergistically activating transcription [60]. Early studies involving co-transfection of an Sp1 expression vector and reporter constructs in *Drosophila* SL2 cells showed that while one Sp binding site gave modest activation of the reporter gene, two sites produced 78-fold greater transcriptional activation [34]. Similar experiments also demonstrated the ability of Sp1 to activate transcription from both proximal and distal sites (using reporter constructs containing sites near the transcription start site or 1.7 kb away), with the presence of both sites eliciting efficient and strong activation of transcription [61]. Electron microscopy revealed that this synergistic activation between proximal and distal sites was achieved through looping of the intervening DNA to allow Sp1 protein interactions [62]. The ability of Sp1 to regulate looping of DNA between enhancers and promoters was more recently confirmed using chromosome conformation capture assays [63]. Furthermore, crosslinking showed that there were interactions between individual Sp1 proteins, with dimers, trimers and tetramers forming both in solution and bound to DNA [34,61]. Additional electron microscopy studies imaged Sp1 as initially forming a tetramer at the promoter site. Upon DNA looping it assembled into multiple tetramers with those at the distal element at the DNA loop junction [64]. This suggests that transcriptional synergy occurs through interaction of Sp1 monomers to form multimer complexes at regulatory elements. Using EMSA and titration of Sp1 protein, it was demonstrated that Sp1 bound initially as a monomer until most of the free DNA template was occupied, followed by a second Sp1 molecule with increasing protein concentration [34]. This shows that the increase in transcription activation is not due to cooperative binding between Sp1 molecules, but rather to synergism, i.e. they do not affect each other's DNA binding affinity, but together can activate transcription to a greater extent than the sum of each alone.

Deletion analysis of the distinct domains of Sp1 revealed that, in addition to the transactivation domains A and B, the C-terminal domain D is required for multimer formation and synergistic transactivation [34]. The domain D deficient Sp1 mutant was able to activate transcription with equal efficiency to wildtype at promoters with a single GC site, but there was a decreased transactivation at promoters with multiple binding sites [61]. For example, the *p21* promoter contains 6 GC boxes and a deletion of domain D in Sp1 gave just a 12-fold increase in transcription of *p21*, in contrast to a 47-fold increase with wildtype Sp1 [65], indicating domain D is required for synergy. Further analysis of the Sp1 domain structure reported that a form of Sp1 unable to bind DNA (missing the zinc finger domain) had no transcriptional activity when expressed alone in SL2 cells, but could interact with a DNA-bound wild type Sp1 protein and significantly enhance transcription, showing superactivation of Sp1 mediated transcription [34,61].

As most members of the Sp/KLF family have similar DNA sequence specificity, yet varying transcriptional stimulation activities, the relative levels of expression of each member in the cell can influence the gene expression pattern [66]. The ratio of Sp1 and Sp3 levels in the cell is particularly important due to their highly similar, indeed almost identical, DNA binding specificity and affinity [21,37]. The relevance of the ratio of the two proteins was highlighted in primary keratinocytes, in which Sp3 levels exceed those of Sp1. However, upon differentiation of the cells *in vitro*, Sp1 levels increase and the Sp3/Sp1 ratio is inverted, suggesting that Sp1 and Sp3 are differentially involved in the regulation of transcription of some cell type-specific genes [29,67]. Sp1 and Sp3 can both cooperate to synergistically activate transcription, such as at the tumor suppressor gene *RASSF1A* [68] and transactivate genes independently, e.g. the gene encoding prostate-specific antigen [68]. However, this is still a contentious issue, as the theory that Sp1 and Sp3 cooperate to regulate transcription is contradictory to findings that Sp1 and Sp3 are present in distinct transcription complexes [33].

Early studies into Sp3 activity reported that Sp3 was not able to initiate expression of several genes with different Sp site-containing promoter elements in *Drosophila* SL2 cells [37]. In fact, Sp3 could repress Sp1 mediated transcription in this system. The repression was dependent on the DNA binding domain, suggesting Sp3 functioned through competition with Sp1 at Sp recognition sites [37]. In light of such studies, it was first thought that Sp3 functioned as a transcriptional repressor molecule. However, co-transfection of Sp1 and Sp3 expression vectors with a number of different Sp1 target genes revealed that only the promoters consisting of multiple GC/GT boxes were subject to Sp3 mediated repression of Sp1 transcription [70]. The ability of Sp3 to repress transcription was found to be due to both the C terminal inhibitory domain (as discussed above) [36] and that Sp3 cannot transactivate synergistically at two or more Sp binding sites [30,71]. Despite Sp3 binding to DNA as a monomer, it can form highly stable complexes with those proteins at nearby Sp recognition sites, which are slower to dissociate than either monomeric Sp3-DNA or multimeric Sp1-DNA complexes. This means Sp3 can outcompete Sp1 for binding at promoters consisting of multiple Sp sites. Moreover, Sp3's inhibitory domain and lack of a domain D like Sp1 means that Sp3 cannot synergistically activate transcription. Thus, when Sp3 displaces the stronger transactivator Sp1 at a regulatory element, there is a net repression of Sp1-mediated transcription [30,60]. This is exemplified by co-transfection studies in *Drosophila*: Sp1 can give about 100 fold increase in *BCAT2* expression, whose promoter has multiple GC boxes, whereas there is only a slight increase with Sp3 [30]. Therefore, the differential expression of different transcription factors and their interplay is important for determining the specific gene expression pattern of a cell.

SP1 REGULATION

While Sp1 is active in all cell types and conditions, it is also tightly regulated enabling Sp1 activity to alter in response to signaling pathways and changing cellular conditions, giving differential expression of inducible and cell cycle/growth genes (including many tumor suppressor genes and oncogenes). One such mechanism is through interactions with other proteins. Transcription factors interact to generate unique patterns of gene expression, meaning the cell can function with a relatively small number of transcription factor proteins. Sp1 has a variety of binding partners dependent on the cell conditions and extracellular signals, which regulate Sp1-dependent transcription [60,72]. Some proteins can bind and enhance Sp1 activity. Oct1, for example, was found to interact with domain B and the adjacent serine/threonine-rich region of Sp1 and increase its DNA binding affinity by cooperatively binding to the distal regulatory element of the U2 snRNA gene to increase transcription [73]. Other proteins can bind to Sp1 and activate transcription synergistically, such as estrogen receptor (ER) proteins. ER binding to Sp1 increases Sp1-DNA binding to estrogen responsive elements independently of estrogen, but the transactivation of the gene is only enhanced in the presence of estrogen [74], illustrating how Sp1-mediated transcription can be altered to respond to signaling pathways. Alternatively, transcription factors can superactivate Sp1-dependent transcription by interacting with DNA-bound Sp1, but not binding to DNA directly, for example AP2, first shown using GAL4 transactivation assays [75].

There are also examples of protein-protein interactions mediating negative effects on Sp1 activity. The cell cycle regulator p53 can bind to Sp1 and interfere with its binding to the *hTERT* promoter (encoding the human telomerase reverse transcriptase gene), thus preventing expression and contributing to tumor suppression [76]. Alternatively, p53 can inhibit expression of the cyclin B1 gene without interfering with Sp1 binding: the inhibition of cyclin B1 was dependent on the Sp1 binding sites and an Sp1/p53 complex was identified at the promoter. However, p53 did not bind the DNA directly, suggesting the inhibition was not through competition/preventing DNA binding, but possibly by disruption of the recruitment of transcription machinery [77]. Conversely, p53 can interact with Sp1 to positively regulate transcription at the *p21* promoter. In proliferating cells, the *p21* promoter is inhibited by HDAC1 binding to Sp1 at the promoter, but upon cell stress and p53 induction, p53 displaces HDAC1 from Sp1 to activate *p21* transcription and halt the cell cycle [52,78].

Sp1 is also highly post-translationally modified, altering Sp1 activity and enabling specific responses to a range of signals. One of the most well studied post-translational modifications of Sp1 is phosphorylation. There are thought to be 23 putative phosphorylation sites in Sp1 and various kinases have been identified, resulting in diverse functional effects [79,80]. Most kinases affect the DNA binding of Sp1, for example, Cyclin B1-Cdk phosphorylates Sp1 at T739 at the C-terminus during mitosis, causing reduced Sp1 DNA binding and facilitating chromatin condensation [81]. In contrast, phosphorylation of S59 at the Sp1 N-terminus by cyclin A-Cdk leads to increased DNA binding and transcription [82]. It can also impact on Sp1 protein stability. During mitosis, JNK phosphorylates T278 and T739, however, with kinase inhibitors to target JNK, Sp1 becomes ubiquitinated and proteasomally degraded [83]. The phosphorylation at T739 was shown to prevent binding to the E3 ubiquitin ligase, thus shielding Sp1 from degradation during mitosis and maintaining levels for cell cycle progression [84].

In addition, Sp1 can be acetylated at K703 in the DNA binding domain, which is linked to its interactions with HAT and HDAC epigenetic regulators. As described above, p300 increases Sp1 binding to DNA, albeit independently of acetylation of Sp1 [49], but acetylation at K703 by p300 reduces their interaction and so decreases the Sp1 transcriptional activity [85]. Sp1 can also be deacetylated by HDAC1, which increases its binding to promoters important for cell cycle and cell death, such as *p21* and *Bak* [86]. Furthermore, Sp1 undergoes various other post-translational modifications, including glycosylation [87,88], poly(ADP-ribosyl)ation [89], methylation [90] and sumoylation [91], which, along with phosphorylation and acetylation modifications, are extensively reviewed in Chu, 2012 [92] and Chang and Hung, 2012 [79].

More recently, several miRNAs have been identified which can post-transcriptionally modulate Sp1 expression, thus providing an additional level of regulation. Examples of such miRNAs are discussed below and reviewed in Safe, 2015 [93]. Together, these different aspects of regulation allow a ubiquitous factor, such as Sp1, to carry out diverse functions in a wide range of cell types.

TISSUE-SPECIFIC ROLES OF SP1

A common theme in gene regulation is the cooperation of ubiquitous transcription factors (including Sp1) with tissue/development stage-specific transcription factors. Much of the research into this mechanism has been performed in the hematopoietic system, one of the most widely studied differentiation systems. Indeed, levels of Sp1 were shown to be high in hematopoietic cells in the mouse embryo [14] and Sp binding sites were identified at many hematopoietic genes [94]. To trigger activation of specific gene programs at certain developmental stages or tissues, Sp1 could either be modified to increase binding/transactivation at a specific site, or Sp1 could bind at the site in only that cell type [95]. These two mechanisms are evident in myeloid differentiation. Firstly, Sp1 undergoes post-translational modification during myeloid spec-

ification. The phosphorylated form of Sp1 increases in myeloid progenitors. This causes increased Sp1 binding to its target site in the promoter for the CD14 cell surface protein, thus giving monocyte-specific promoter activity [96]. Secondly, epigenetic changes could alter the availability of the binding site. The myeloid transcription factor Pu.1 binds close to the Sp1 site at the integrin *CD11b* promoter. Pu.1 binding exposes the binding site for Sp1 in the chromatin, allowing it to bind and regulate transcription in a tissue-specific manner [97].

In addition, Sp1 can interact with tissue-specific transcription factors to generate tissue-specific gene expression programs. In erythroid cells, Sp1 can cooperate synergistically with the transcription factor GATA1 at erythroid-specific promoters. Sp1 and GATA1 binding sites can be seen in close proximity at many promoters and enhancers of erythroid-specific genes. The two proteins physically interact at the DNA binding domains to synergistically activate transcription, for example at the *EpoR* promoter [98], the *Tal1* promoter [99] and the *ALAS2* gene (required for heme synthesis), where they also recruit the activator p300 [100]. Sp1/GATA1 complexes have also been identified at promoters without GATA sites, suggesting Sp1 can recruit tissue-specific transcription factors to particular regulatory elements [98]. Furthermore, Sp1 and GATA1 could interact from a distance in reporter constructs modelling the architecture of globin locus control regions, suggesting the two proteins can interact to stabilize loops between regulatory regions and synergistically activate the globin gene [98]. SCL/TAL1 is an important regulator of hematopoietic specification. It forms a complex with many other proteins (e.g. LMO2, Ldb1, E2A), which has been reported to enhance *Kit* expression, encoding a receptor needed in hematopoiesis. The complex is tethered to the promoter by Sp1, with the interaction between the cell type-specific factors and a ubiquitous transcription factor determining the gene expression profile and cell fate [94]. These studies also demonstrate that Sp1 can recruit SCL and GATA1, plus other restricted transcription factors, to specific promoters, but not to all, indicating that the promoter architecture is also important in regulation of tissue-specific genes, likely to position the proteins to enable functional interactions [94].

Despite Sp1 knockout causing embryonic lethality in mice, Sp1-deficient (with a knockout of the DNA binding domain) ESCs could grow normally in culture [11]. During *in vitro* differentiation to mimic embryonic hematopoiesis in culture, Sp1-deficient embryonic stem cells could proceed through most stages of blood cell development, but Sp1 was required for terminal differentiation. Gene expression analysis of purified cells representing successive stages of hematopoietic specification revealed a progressive deregulation of gene expression: most Sp1 target genes were unaffected, but Cdx and some Hox genes were downregulated at an early stage, and the number of affected genes increased through later stages as a result, causing a failure in terminal hematopoietic differentiation. Interestingly, the deletion of Sp1 at later developmental stages, in this case at the myeloid progenitor stage had no effect, indicating that the defects in the Sp1 knockout mice were cumulative [101].

Additional tissue- and developmental-specific roles have been discovered for Sp1 and other Sp factors, including in the nervous system. The *NR1* gene encodes an essential component of the *N*-methyl-D-aspartate receptor, which is important for neuronal differentiation. Sp factors bind to and activate an NFκB site in the promoter to upregulate *NR1* expression: specifically Sp3 during neuronal differentiation and Sp1 in differentiated neuronal cells [102]. Sp1, Sp3 and Sp4 interact to activate neuronal-specific transcription of cyclin-dependent kinase 5/p35, which is critical for brain function [103]. However, Sp3 and Sp4 together repress expression of superoxide dismutase 2 in neurons, but the substitution of Sp4 for Sp1 in astroglia causes upregulation of transcription [104]. Moreover, Sp1 and Sp3 mediate expression of cyclooxygenase-2 in response to oxidative stress in neurons to aid in neuronal survival [105]. This highlights the importance of the balance between the levels and activities of related Sp transcription factors in the function of the nervous system, particularly the tissue-specific Sp4 and ubiquitous Sp1 and Sp3.

These examples demonstrate the ability of ubiquitously expressed factors to contribute to lineage specific regulatory programs and highlights important general principles in developmentally controlled gene regulation.

SP1 IN DISEASE

Given the role of Sp1 in a multitude of cellular pathways and processes, it is unsurprising that it is associated with the pathogenesis of a number of diseases, with perhaps the best studied being cancer. Sp1 overexpression is seen in a host of cancer cell types, where levels of Sp1 also correlate with tumor stage and a poor prognosis [12]. Knockdown of Sp1 in cancer cell lines (including breast, kidney, pancreatic, lung, and colon cancers) led to decreased survival and the inhibition of cell growth and migration. Similarly, tumor formation and metastasis was reduced in mouse xenograft models with Sp1 knockdown. Furthermore, the changes in gene expression following knockdown correlated with the observed phenotypic changes of the cells [106]. Indeed, several anticancer agents in clinical use act by inhibiting Sp1 action [107]. Mithramycin A (and its analogues) can alter the binding of Sp1 to DNA and downregulate Sp1-mediated transcription [108,109]. Tolfenomic acid increases the ubiquitination and degradation of Sp1 [110], while anthracyclines, one of the most effective anticancer treatments, bind DNA at GC-rich sequences, preventing Sp1 binding [111,112], though this may not be its sole mechanism of action. Other

drugs can act on Sp1 indirectly, such as curcumin, which increases reactive oxygen species in the cell, causing activation of ZBTB4/10 proteins that displace Sp1 from GC-rich sites and decreased Sp1 expression [113].

The role of Sp1 in cancer stems from its regulation of genes that are involved in all of the hallmarks of cancer: growth factor-independent proliferation, immortality, evasion of apoptosis, angiogenesis, tissue invasion and metastasis [72,114]. Sp1 is involved in the regulation of genes required for the progression of the cell cycle and entry into S-phase, such as cyclins and *MYC*, as well as in growth factor signaling pathways e.g. *IGF1R* has up to eight Sp sites at its promoter and IGF signaling is commonly used by cancer cells to maintain proliferation [115,116]. However, it also regulates the transcription of cell cycle inhibitor genes, for example, synergizing with p53 under conditions of cell stress to activate transcription of *p21* [65]. Sp1 regulates the expression of telomerase subunits involved in the maintenance of telomeres and cell immortality. It can bind to five Sp sites present at the *hTERT* promoter to activate gene expression [117], or conversely, interact with HDACs to repress *hTERT* expression [57]. Sp1 is involved in the control of both pro- and anti-apoptotic factors, which have a direct role in cancer development. Survivin is a protein that promotes cell survival by inhibiting apoptosis and is essential in many tumors: its overexpression is directly associated with an increase in levels of Sp1 [118]. The pro-angiogenic factor VEGF has Sp1 binding sites at its promoter: estrogen signaling in breast cancer can result in interaction of Sp1 with ERα and subsequent upregulation of the *VEGF* gene [119]. Sp1 is also involved in maintaining genome stability via regulation of DNA damage factors and inflammatory signaling to drive oncogenesis [72].

While the deregulation of signaling pathways and transcription factor networks has been well studied, the impact of aberrantly expressed miRNAs in cancer is a newly developing field. Specific miRNAs have been found to be downregulated in certain cancers, such as miRNA223 in gastric cancer [120]. In this example, Sp1 protein levels were also found to increase, but with no change in mRNA levels, suggesting post-transcriptional regulation. miRNA223 was found to bind to the 3' untranslated region of Sp1 mRNA and inhibit its translation. The increase in Sp1 led to enhanced epithelial-mesenchymal transition (EMT), involved in promoting cell migration and invasion in tumorigenesis, whereas overexpression of miRNA-223 in a gastric cancer model caused decreased EMT and proliferation, and induced apoptosis [120]. A similar action was discovered for miRNA-324-5p in Hepatocellular carcinoma (HCC) [121] and miRNA-23b in multiple myeloma [122]. Additionally, Sp1 has been found to regulate the expression of miRNAs. miRNA-195 promotes cell apoptosis and suppresses cancer cell proliferation/metastasis; its expression is frequently reduced in various cancers. Characterization of its promoter region found an Sp1 site required for *miRNA-195* expression, but in HCC cells, Sp1 interacted with HDAC3 at the promoter to repress transcription [123]. The miRNA-23a-27a-24-2 cluster is deregulated in many cancers. The promoter, containing 2 Sp1 sites, was found to be demethylated in Hep2 cells, compared to control HEK293 cells, leading to upregulation of the cluster and promotion of proliferation and cell survival of cancer cells [124]. Furthermore, Sp1 was found to be involved in a regulatory network with another transcription factor (NFκB), an epigenetic regulator (HDAC) and a miRNA (miRNA-29b) to modulate *KIT* expression in a subset of acute myeloid leukemia (AML) [125]. miRNA-29b acts to post-transcriptionally inhibit Sp1. Conversely, Sp1, along with NFκB, binds to the *miRNA-29b* enhancer and interacts with HDAC1/3 to form a repressive complex and inhibit *miRNA-29b* expression. Aberrant activation of *KIT* in AML cells leads to upregulation of *MYC*, which in turn results in downregulation of *miRNA-29b* and an increase in Sp1 expression. Sp1, along with NFκB, activates *KIT* transcription, thus completing the regulatory loop and contributing to the disease state [125].

Sp1 can be linked to the changes in DNA methylation often observed in cancer cells. Sp1 can be involved in the protection of regulatory regions of genes (especially housekeeping genes) from methylation [55,56] and when methylation spreads to Sp1 sites, binding is inhibited, contributing to gene silencing [126]. Sp1 mediates transcription of the tumor suppressor *RASSF1A*, whose promoter has four Sp sites. In cancer, a change in histone modifications (H3 deacetylation and K9 trimethylation) causes a reduction in Sp1 binding, followed by methylation of the promoter and gene silencing [127]. This suggests that the increase in Sp1 levels in cancer cells is not sufficient to overcome the silencing of its target genes through DNA methylation. However, Sp1 can also interact with DNMT1 to promote methylation at specific sites [53,54], suggesting a role for Sp1 in establishing the epigenetic state of both normal and cancer cells. More studies are needed to completely understand its mechanism in transcription activation and epigenetics. The ability of Sp1 to regulate oncogenes and tumor suppressors, pro-survival and pro-apoptotic genes, highlights the need to fully understand Sp1's activity at different promoters and in different cell conditions to develop a therapy that can specifically target Sp1 in cancer.

Sp1 has been implicated in Huntington's disease, a dominantly inherited neurodegenerative disorder caused by expansion of a polyglutamine tract in the Huntingtin (Htt) protein. Htt was found to bind to Sp1 and TAFII130 and inhibit DNA binding, while overexpression of both factors in striatal cells from a mouse model of Huntington's led to an improvement of symptoms and reversed inhibition of the dopamine D2 receptor gene, known to be a marker of the disease [128]. Further studies suggested the protective role of Sp1 overexpression involved acti-

vation of cystathione γ-lyase gene expression, the biosynthetic enzyme for cysteine, which is depleted in disease tissues [129]. However, this issue is still disputed, as other studies have found Sp1 contributes to the pathology in Huntington's disease. Sp1 was found to be overexpressed in the brains of mouse models and in model cell lines: inhibition or knockout of Sp1 led to amelioration of toxicity caused by mutant Htt and the mice survived longer, possibly due to Sp1 negatively regulating the Dopamine D2 gene. This suggests Sp1 is a potential therapeutic target in Huntington's disease [130].

A positive role of Sp1 has been found in Alzheimer's disease, where inhibition of Sp1 with mithramycin A in transgenic mouse models led to further memory impairment and an increase in the levels of Amyloidβ peptides (a major hallmark of the disease) [131]. A polymorphism in an Sp1 binding site of the *COL1A1* gene, encoding collagenα1, a major protein in bone, is associated with a predisposition to osteoporosis by altering the ratio of collagen α1 to α2 chains, causing reduced biomechanic strength in the bones [132]. In contrast, there was a negative correlation between the same polymorphism and hip osteoarthritis, suggesting there is a reduced risk of the disease [133]. Furthermore, Sp1 has been implicated in the development of multiple sclerosis (MS). Polymorphisms in the *IRF5* and *CD24* genes, factors involved in MS, can lead to increased Sp1 binding at these genes and an increased risk of MS [134,135]. Gene expression analysis in MS patients suggested the involvement of Sp1 in gender-specific gene signatures and inhibition of Sp1 transcription reduced the incidence and severity of experimental autoimmune encephalomyelitis in mice (the model of MS), highlighting Sp1 as a potential therapeutic target in MS [136].

CONCLUSION

Sp1 was the first characterized and still is one of the best studied mammalian transcription factors. It functions as a transcriptional activator of a variety of genes including house-keeping genes, cell cycle regulators and tissue-restricted genes. It is ubiquitously expressed, but its activity can be modified to respond to external stimuli, different stages of the cell cycle and different cell functions through post-translational modification and interaction with other transcriptional regulators. It can also regulate tissue- and developmental stage-specific gene expression, but there is still little known about the protein interactions and/or post-translational modifications that occur to elicit the specific patterns of Sp1-mediated gene expression. Therefore, more work is needed to both further understand the role of ubiquitous transcription factors in tissue-specific gene regulation and the dynamic transcription network controlling cell specification. This review highlights the importance of models of development to elucidate the mechanism of transcription activation, as well as the need to further our understanding of Sp1-mediated transcription in development and disease.

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